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Role of the β-Lactamase of *Campylobacter jejuni* in Resistance to β-Lactam Agents

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We studied the role of the β-lactamase of *Campylobacter jejuni* in resistance to β-lactam agents. β-Lactamase-positive strains were more resistant than β-lactamase-negative strains to amoxicillin, ampicillin, and ticarcillin ($P < 0.05$). With penicillin G, piperacillin, imipenem, and six cephalosporins, the susceptibility levels were similar for both β-lactamase-positive and -negative strains. By using spectrophotometric and microbiological assays, the β-lactamase from three strains hydrolyzed ampicillin, amoxicillin, penicillin G, cloxacillin, and, partially, cephalothin. Ticarcillin and piperacillin were partially hydrolyzed in the microbiological assay. There was no activity against five other cephalosporins or imipenem. Isoelectric focusing of the enzyme showed a pI of 8.8. Tazobactam was the best inhibitor of the enzyme, followed by clavulanic acid, sulbactam, and cefoxitin, while EDTA and *p*-chloromercuribenzoate had no activity. All β-lactamase-positive strains became susceptible to amoxicillin and ampicillin with 1 μg of clavulanic acid per ml. With the same inhibitor, there was a reduced but significant effect for ticarcillin but no effect for penicillin G or piperacillin. Sulbactam had no effect and tazobactam was effective only at 2 μg/ml on amoxicillin and ampicillin. The β-lactamase of *C. jejuni* seems to be a penicillinase with a role in resistance for only amoxicillin, ampicillin, and ticarcillin.

Campylobacter jejuni has been recognized as a major cause of human bacterial diarrhea, and from 83 to 92% of the strains are β-lactamase producers (12, 23). Although antibiotic susceptibility testing is not yet standardized for *Campylobacter* species, the pattern in β-lactam susceptibility seems to be well established. All strains of *C. jejuni* are resistant to cefoxitin, cefamandole, and cefoperazone (1, 9, 10, 12, 25), and most or all strains are resistant to cephalothin and cefazolin (9, 10, 12, 15, 25–28). The level of resistance is variable for penicillin G, ampicillin, amoxicillin, ticarcillin, piperacillin, cefotaxime, and moxalactam (6, 10, 12, 27), but all strains are susceptible to imipenem (1, 12). The purpose of this study was to investigate the role of the β-lactamase in the resistance of *C. jejuni* to various β-lactam agents.

MATERIALS AND METHODS

Bacterial strains. From a collection of 160 human strains of *C. jejuni* isolated in five hospitals in the Montreal area (12), 20 of the strains that were most resistant to ampicillin, all of which were β-lactamase positive, and all 13 β-lactamase-negative strains were chosen for use in this study. The strains were identified as *C. jejuni* by standardized methods (16). The strains were preserved in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 15% (vol/vol) glycerol and were stored at −70°C.

Antibiotics. Antimicrobial agents were kindly provided by the indicated manufacturers, as follows: tazobactam (American Cyanamid Co.); amoxicillin trihydrate, cloxacillin sodium, and penicillin G potassium (Ayerst Laboratories Canada); clavulanic acid and ticarcillin disodium (Beecham Laboratories Canada); cefamandole nafate, cephalothin sodium, and moxalactam diammonium (Eli Lilly Canada Inc.); cefuroxime sodium and cephaloridine (Glaxo Ltd. Canada); piperacillin sodium (Lederle Co.); cefoxitin sodium and imipenem (*N*-formimidoylthienamycin monohydrate) (Merck Sharp & Dohme Research Canada, International); ampicillin trihydrate, cefoperazone sodium, and sulbactam (Pfizer Inc. Canada); and cefotaxime sodium (Roussel Canada Inc.). Nitrocefin was purchased from Oxoid Canada Inc.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was done by an agar dilution method as described by Gaudreau et al (5). The concentrations (in micrograms per milliliter) of β-lactam antibiotics tested were 0.0625 to 128 for amoxicillin, ampicillin, penicillin G, piperacillin, and ticarcillin; 0.125 to 64 for cefotaxime, imipenem, and cefoperazone; 0.25 to 512 for cefamandole; 1 to 256 for cefoxitin; and 2 to 256 for cephalothin and moxalactam. Inocula were prepared in Mueller-Hinton broth (BBL Microbiology Systems) that was directly inoculated with a fresh culture from a blood agar plate to a density adjusted to approximately a no. 0.5 McFarland turbidity standard and diluted 1:10. The suspension was distributed into the wells of a Steers replicator apparatus. A 1-μl volume of each suspension corresponding to a final inoculum of approximately 10^4 CFU was inoculated onto the antibiotic medium. The medium used was Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). The inoculated plates were incubated at 35°C under a microaerophilic atmosphere for 48 h. The endpoint was taken as the complete inhibition of macroscopic growth. The following control strains were inoculated onto each plate: *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, and *Escherichia coli* ATCC 25922. Susceptibility criteria were those of the National Committee for Clinical Laboratory Standards (17).

Detection of β-lactamase. The β-lactamases were detected by use of cefinase disks (BBL Microbiology Systems). The procedures of the manufacturer were followed. *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as positive and negative controls. A designation of 2+ was

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noted when a red color was obtained in 15 min, and a designation of 1+ was noted when a red color was obtained in 30 min. The absence of a red color after 30 min was considered negative.

β-Lactamase extraction. The three *C. jejuni* strains with the most rapid reaction on the cefinase disks were selected for extraction: strain 52, strain 138, and strain 160. They were first cultivated on blood agar for 24 h at 42°C under a microaerophilic atmosphere by using a gas generator envelope (Oxoid Canada, Nepean, Ontario, Canada). A suspension of bacteria was obtained in brucella broth (BBL Microbiology Systems) to a density approximating a McFarland no. 5 turbidity standard. One milliliter of this suspension was added to 50 ml of brucella broth in a 250-ml flask sealed with a porous stopper, which was placed in a jar under microaerophilic conditions. The jar was incubated in a Lab-Line orbit environ-shaker (Lab-Line Instruments Inc.) at 42°C and shaken at 150 rpm for 24 h. The incubation period was determined in preliminary assays with the three strains. β-Lactamase production was monitored during the growth phase by regularly sampling the broth and measuring enzyme activity, after extraction, by the methods outlined below. With these assays, a 24-h incubation period was found to yield the highest activity of the β-lactamase for the three strains. After incubation, cellular counts were approximately 3×10^{11} CFU/liter, with an optical density of between 0.46 and 0.52 at 600 nm, which is roughly equal to a no. 2 McFarland turbidity standard. For extraction, 380 ml of broth was centrifuged and cells were harvested and washed twice in phosphate buffer (0.05 M; pH 7.0), suspended in a final volume of 20 ml of the same buffer, and broken in a French pressure cell (Aminco SLM Instruments Inc.). The lysed cell extract was centrifuged at 22,000 × *g* for 20 min at 4°C. The supernatant was pooled and used as the crude β-lactamase.

β-Lactamase assay. Cephalosporinase activity was measured by the method of O'Callaghan et al. (20) by using a UV/VIS spectrophotometer (Lambda 4B; Perkin-Elmer) with cuvettes with a path of 1 cm. Wavelengths of 259 nm for cephaloridine, 262 nm for cephalothin, 274 nm for cefamandole, 265 nm for cefuroxime, 267 for cefoxitin, 261 nm for cefotaxime, 297 nm for imipenem, and 482 nm for nitrocefin were used (19). For the penicillins, the microiodometric assay of Sykes and Nordström (24) was used. Specific activities were determined in at least duplicate experiments, with standard deviations of less than 10%.

Microbiological assay. Hydrolysis of β-lactam antibiotics was confirmed in a microbiological assay. A volume of 0.1 ml of a standardized inoculum of *Bacillus subtilis* ATCC 6633 (Difco Laboratories) spores was incorporated into 50 ml of Mueller-Hinton agar (BBL Microbiology Systems) and poured into petri dishes (150 by 15 mm; Phoenix Biomedical Products) with 4-mm wells cut in the agar. The final concentration of spores in the agar was 10^6 CFU/ml. For each antibiotic tested, 0.5 ml of the *C. jejuni* β-lactamase extracts was incubated with 0.5 ml of the β-lactam at 10 μg/ml for 15 min. After that period, the reaction was stopped by the addition of methanol. Wells were filled with 25 μl of the reaction mixture, and the plates were incubated at 37°C for 18 h. The resulting concentrations of active antibiotic were estimated by comparison of the zone sizes produced around the test wells with those obtained around control wells containing standard antibiotic concentrations without β-lactamase. The precision and accuracy of the microbiological assay were evaluated by the repeatability of 10 diameter measurements of standard antibiotic concentrations.

Inhibition studies. The following inhibitors were tested: cefoxitin, 10 μM; EDTA, 10 and 100 μM (Fisher Scientific Co.); and *p*-chloromercuribenzoate, 10 and 100 μM (Sigma Chemical Co.). Enzyme preparations were exposed to the inhibitors for 10 min at 37°C prior to the addition of nitrocefin (final concentration, 100 μM). The percentage of inhibition was determined spectrophotometrically by comparing enzyme activities with and without the inhibitor. For clavulanic acid, sulbactam, and tazobactam, the concentration that inhibited 50% of the enzyme activity (I_{50}) was calculated by regression analysis. The following procedure was used: 0.5 ml of the inhibitor was incubated at 37°C for 10 min with 0.5 ml of the β-lactamase diluted in phosphate buffer to a final concentration that was able to destroy 1 ml of nitrocefin (100 μM) in 5 min.

Isoelectric focusing. Isoelectric focusing was performed at 4°C on prepared LKB Ampholine polyacrylamide gels with pHs of 3.5 to 9.5 for the three extracts and for 15 other strains of *C. jejuni*, all of which were nitrocefin positive. Only one gel was used, but two series of the same extract were focused at the same time. One series was applied on the top half of the gel; the other was applied on the lower half. After isoelectric focusing, the gel was cut into two halves. One was put in the nitrocefin solution at 37°C for 5 min, and the other one was stained with Coomassie blue. Before isoelectric focusing, LKB pI markers were applied on the lower part of the gel (the half to be stained). The bands stained by Coomassie blue were the same as those revealed by the nitrocefin solution.

Effect of β-lactamase inhibitors on susceptibility to penicillins. Antibiotic susceptibility testing was done by the same agar dilution method described above. Twofold dilutions of each drug were tested, with a fixed concentration of a β-lactamase inhibitor added in each plate. The concentrations (in milligrams per liter) of penicillins were the same as those used for antibiotic susceptibility testing. Clavulanic acid was added to final concentrations of 0.5 and 1 μg/ml, whereas sulbactam and tazobactam were used at 0.5 and 2 μg/ml. An equivalent volume of distilled water was added to the last set of plates. Each β-lactamase inhibitor was tested with the full range of antibiotic dilutions. A control plate without antibiotic and another one with the β-lactamase inhibitor alone were inoculated in each series. The MICs of each β-lactamase inhibitor were determined by the same method with twofold dilutions from 0.12 to 16 μg/ml.

RESULTS

Antibiotic susceptibility testing. The susceptibilities of the 33 strains of *C. jejuni* to β-lactam antibiotics are presented in Table 1. The β-lactamase-positive strains were significantly more resistant to amoxicillin, ampicillin, and ticarcillin ($P < 0.05$; Mann-Whitney U test) than the β-lactamase-negative strains were. All β-lactamase-positive and -negative strains were resistant to cephalothin, cefamandole, cefoxitin, and cefoperazone. The 33 strains were susceptible to imipenem. With piperacillin, penicillin G, and moxalactam, all strains were resistant except for five, three, and three strains, respectively, which were moderately susceptible. For cefotaxime, there was no significant difference in the susceptibilities of the β-lactamase-positive and -negative strains ($P > 0.05$; Mann-Whitney U test).

β-Lactamase activity. Table 2 presents the activity profiles of the *C. jejuni* β-lactamase extracts against penicillins and cephalosporins. By the spectrophotometric assay, none of the cephalosporins tested were hydrolyzed. By the micro-

TABLE 1. Detection of β-lactamase by the nitrocefin test and susceptibility of *C. jejuni* to β-lactam antibiotics

Positivity with nitrocefin[a]	No. of strains	MIC range (μg/ml)[b]										
		Peni G	Amp	Amx	Tic	Pip	Cth	Cfm	Cefox	Cefop	Cefot	Moxa	Imi
2+	4	64–128	32–64	32–64	32–128	>128	>64	>64	>64	>64	8–32	≥64	0.25–0.5
1+	16	32–128	4–64	4–64	16–128	≥128	>64	>64	>64	>64	16–64	32–>64	0.25–0.5
−	13	8–128	2–16	0.5–8	8–128	64–>128	>64	>64	>64	>64	4–32	32–>64	<0.125–1

[a] 2+, positive in 15 min; 1+, positive in 30 min; −, negative.
[b] Peni G, penicillin G; Amp, ampicillin; Amx, amoxicillin; Tic, ticarcillin; Pip, piperacillin; Cth, cephalothin; Cfm, cefamandole; Cefox, cefoxitin; Cefop, cefoperazone; Cefot, cefotaxime; Moxa, moxalactam; Imi, imipenem.

iodometric assay, ampicillin was hydrolyzed more than penicillin G, amoxicillin, and cloxacillin were. The *C. jejuni* 52 extract hydrolyzed amoxicillin better than cloxacillin did, but the activities of the two other extracts against these substrates were quite similar. The substrate profiles of the extracts were also studied by the microbiological assay. With penicillin G, ampicillin, and amoxicillin, there was no zone of inhibition after exposure to each β-lactamase extract. With ticarcillin, the residual antibiotic activity was 17, 18, and 54% for *C. jejuni* 52, 160, and 138 extracts, respectively. With piperacillin, the activities were 63, 65, and 60%, respectively. In contrast, for cephalothin, the residual activities were 85, 83, and 80%, respectively; and for cephaloridine and cefotaxime, they were 100% for each extract. The precision of the microbiological assay was evaluated, and the measured diameters varied ±4.8% for ampicillin, ±4% for penicillin G, ±5.4% for amoxicillin, ±5% for ticarcillin, ±5.1% for piperacillin, ±4.9% for cephalothin, ±6.8% for cefotaxime, and ±2.1% for cephaloridine.

Inhibition studies. At 10 μM, the percentages of inhibition for cefoxitin were 9.5, 10.4, and 3.2 for *C. jejuni* 52, 138, and 160, respectively, while EDTA and *p*-chloromercuribenzoate at 10 and 100 μM had no inhibitory effect on β-lactamase activity. In Table 3, the calculated I_{50}s are presented. The most potent inhibitor was tazobactam, followed by clavulanic acid and sulbactam.

Isoelectric focusing. Isoelectric focusing revealed only one β-lactamase band with a pI of 8.8 for the extracts of the 3 *C. jejuni* strains and for 15 other strains tested.

Effect of β-lactamase inhibitors on susceptibility to penicillins. The range of clavulanic acid MICs for 33 strains was 1 to 4 μg/ml, with an MIC for 50% of strains tested of 2 μg/ml and an MIC for 90% of strains tested of 4 μg/ml. The range of sulbactam MICs was 4 to >16 μg/ml, with an MIC for 50% of strains tested of 16 μg/ml and an MIC for 90% of strains tested of >16 μg/ml, and the MICs of tazobactam were all >16 μg/ml. As shown in Table 4, all the β-lactamase-positive strains became susceptible to amoxicillin with 0.5 μg of clavulanic acid per ml except for two of the strongest β-lactamase producers (strains 52 and 48, for which the clavulanic acid MIC was 2 μg/ml each). These strains became susceptible with the addition of 1 μg of this inhibitor per ml. With ampicillin and ticarcillin, 1 μg of clavulanic acid per ml had a significant effect on the susceptibilities of β-lactamase-positive strains. Tazobactam at a concentration of 2 μg/ml, but not at a concentration of 0.5 μg/ml, increased significantly the susceptibilities of these strains to amoxicillin and ampicillin. No significant effect was noted with the association of clavulanic acid and penicillin G or piperacillin, sulbactam, and the five penicillins tested and tazobactam with penicillin G, piperacillin, or ticarcillin. The β-lactamase inhibitors had no significant effect on the susceptibilities of the β-lactamase-negative strains to the five penicillins tested (data not shown).

TABLE 2. β-Lactamase activity of *C. jejuni* extracts against penicillins and cephalosporins[a]

β-Lactam	Activity (nmol of substrate hydrolyzed/min/mg of protein) of the following *C. jejuni* strain:		
	52	138	160
Penicillin G	184.1	95.8	103.7
Ampicillin	230.9	142.2	147.0
Amoxicillin	147.3	56.2	73.2
Cloxacillin	109.0	66.4	82.0
Cephaloridine	NA[b]	NA	NA
Cephalothin	NA	NA	NA
Cefamandole	NA	NA	NA
Cefuroxime	NA	NA	NA
Cefoxitin	NA	NA	NA
Cefotaxime	NA	NA	NA
Imipenem	NA	NA	NA
Nitrocefin	93.0	41.0	88.0

[a] Penicillins were tested by the microiodometric assay, and cephalosporins (100 μM) were tested by the spectrophotometric assay.
[b] NA, no activity (too weak to be measured).

DISCUSSION

C. jejuni has a particular pattern of β-lactam resistance, and between 83 and 92% of the strains produce a β-lactamase. In this study, we partially characterized the β-lactamase and evaluated its role in β-lactam resistance. When compared with the β-lactamase-negative strains, the β-lactamase-positive strains were significantly more resistant to amoxicillin, ampicillin, and ticarcillin. For both β-lactamase-positive and -negative strains, resistance to penicillin G, piperacillin, imipenem, and six cephalosporins was similar. Fleming et al. (4) and Maia et al. (14) reported an association between the β-lactamase production of *C. jejuni* and ampicillin and amoxicillin resistance, respectively.

For enzymatic studies, we first determined the optimal conditions for β-lactamase production. Shaking of the cul-

TABLE 3. I_{50}s calculated for β-lactamases

Inhibitor[a]	I_{50} (μM) for β-lactamases from the following *C. jejuni* strain:		
	52	138	160
CLA	45	43	36
SUL	59	71	48
TAZ	1.5	1.5	1.4

[a] CLA, clavulanic acid; SUL, sulbactam; TAZ, tazobactam.

TABLE 4. Susceptibilities of *C. jejuni* β-lactamase-positive strains to penicillins in combination with β-lactamase inhibitors

Antimicrobial agent	β-Lactamase inhibitor (concn [μg/ml])[a]	No. of strains tested	MIC (μg/ml)[b]			No. of strains in category[c]:			
			Range	50%	90%	S	M	R	P^d
Amoxicillin	No inhibitor	20	4–64	32	64	8	0	12	
	Clav (0.5)	20	1–16	4	8	18	2	0	<0.05
	Clav (1)	19	1–8	2–4	4	19	0	0	<0.05
	Sulb (0.5)	20	2–64	16	64	8	2	10	>0.05
	Sulb (2)	20	4–64	16	32	7	2	11	>0.05
	Tazo (0.5)	20	2–32	16	16	9	9	2	>0.05
	Tazo (2)	20	1–32	8	16	15	3	2	<0.05
Ampicillin	No inhibitor	20	4–64	32	64	2	6	12	
	Clav (0.5)	20	4–32	32	32	4	4	12	>0.05
	Clav (1)	19	1–8	8	8	19	0	0	<0.05
	Sulb (0.5)	20	2–32	16	32	3	9	8	>0.05
	Sulb (2)	20	4–32	16	32	3	10	7	>0.05
	Tazo (0.5)	20	4–64	32	32	2	4	14	>0.05
	Tazo (2)	20	4–32	16	16	8	10	2	<0.05
Penicillin G	No inhibitor	20	32–128	64	128	0	0	20	
	Clav (0.5)	20	8–128	32	128	0	2	18	>0.05
	Clav (1)	19	16–128	32	64	0	0	19	>0.05
	Sulb (2)	20	16–128	64	128	0	0	20	>0.05
	Tazo (2)	20	16–128	64	128	0	0	20	>0.05
Piperacillin	No inhibitor	20	128–>128	>128	>128	0	0	20	
	Clav (0.5)	20	32–>128	128	>128	0	4	16	>0.05
	Clav (1)	19	64–>128	128	>128	0	1	18	>0.05
	Sulb (0.5)	20	64–>128	>128	>128	0	1	19	>0.05
	Sulb (2)	20	32–>128	>128	>128	0	0	20	>0.05
	Tazo (0.5)	20	64–>128	>128	>128	0	1	19	>0.05
	Tazo (2)	20	64–>128	>128	>128	0	0	20	>0.05
Ticarcillin	No inhibitor	20	8–128	64	128	2	10	8	
	Clav (0.5)	20	4–128	32	64	5	13	2	>0.05
	Clav (1)	19	4–32	16	32	16	3	0	<0.05
	Sulb (0.5)	20	8–128	64	128	4	11	5	>0.05
	Sulb (2)	20	8–>128	64	128	4	9	7	>0.05
	Tazo (0.5)	20	8–128	32	128	3	14	3	>0.05
	Tazo (2)	20	8–128	64	128	2	12	6	>0.05

[a] Clav, clavulanic acid; Sulb, sulbactam; Tazo, tazobactam.
[b] 50% and 90%, MIC for 50 and 90% of strains, respectively.
[c] Breakpoints are those of the National Committee for Clinical Laboratory Standards (17). S, susceptible (≤8 μg/ml for amoxicillin and ampicillin, ≤1.5 μg/ml for penicillin G, and ≤16 μg/ml for piperacillin and ticarcillin); M, moderately susceptible (16 μg/ml for amoxicillin and ampicillin, 2 to 8 μg/ml for penicillin G, and 32 to 64 μg/ml for piperacillin and ticarcillin); R, resistant (≥32 μg/ml for amoxicillin and ampicillin, ≥16 μg/ml for penicillin G, and ≥128 μg/ml for piperacillin and ticarcillin).
[d] Compared with no inhibitor (Mann-Whitney U test).

ture during incubation gave more rapid growth, and the highest β-lactamase production by the three strains was obtained after 24 h. By spectrophotometric, microbiological, or both assays, the β-lactamase extracts had a penicillinase activity profile that hydrolyzed the six penicillins tested, nitrocefin but not imipenem, and five of the six cephalosporins tested. This profile is similar to the one reported by Lucain et al. (13) in 18 of 21 β-lactamase-producing strains. Using a bioassay, they noted hydrolysis for penicillin G, ampicillin, oxacillin, and carbenicillin; weak hydrolysis for cephalothin; but no activity against cephaloridine, cefuroxime, or cefotaxime. The pI of our extracts was 8.8, while Lucain et al. (13) found a pI of 8.3. Also, they suggested the presence of another type of β-lactamase for each of three other strains on the basis of slight differences in the activity profiles against cephalosporins; isoelectric focusing, with pIs ranging from 7.4 to 8.6; and molecular weight determinations.

In the inhibition studies, tazobactam was the strongest inhibitor of β-lactamase, followed by clavulanic acid and sulbactam. In contrast, clavulanic acid was the best inhibitor of amoxicillin, ampicillin, and ticarcillin by susceptibility testing. Gutmann et al. (8) observed in β-lactamase-producing *E. coli* that the better activity of clavulanic acid compared with that of tazobactam was not due to a better enzyme-inhibitory effect but to its intrinsic activity or better penetration rate. Chin and Neu (3) also suggested that tazobactam enters the cells of gram-negative bacteria less well than clavulanate does. In view of our results, this also could be the case for *C. jejuni*. For the inhibitory effect of clavulanic acid, our results are in agreement with those of Maia et al. (14) and Gaudreau et al. (5). However, Van der Auwera and Scorneaux (25) and Segreti et al. (22) found no effect of clavulanic acid on the susceptibility of *C. jejuni* to ampicillin and amoxicillin. These discrepancies could possibly be explained by the fact that different methodologies

were used. In our study, sulbactam was not effective at 0.5 or 2 μg/ml, which is in agreement with the inhibition results. Other groups of investigators (2, 7, 11) also found that sulbactam was less effective than clavulanic acid or tazobactam when combined with β-lactams in other gram-negative species.

In this study, we found a significant association with the presence of β-lactamase and resistance to ampicillin and amoxicillin; clavulanic acid brought the susceptibility levels of β-lactamase-positive strains to those of β-lactamase-negative strains. Indeed, the extracts hydrolyzed those agents. With ticarcillin, there was partial hydrolysis with the extracts. The effect of clavulanic acid at 1 μg/ml remained significant but less pronounced. Although β-lactamase-positive strains were significantly more resistant to ticarcillin, the MICs for four β-lactamase-negative strains were 32 μg/ml or higher. These findings indicate the possibility of another mechanism of resistance to ticarcillin besides β-lactamase. Although the β-lactamase extracts hydrolyzed penicillin G and piperacillin, there was no effect of clavulanic acid on their MICs and no difference in the susceptibilities of β-lactamase-positive and -negative strains. These results suggest that penicillin G and piperacillin penetrate the bacterial cell poorly or may have differential affinities for penicillin-binding proteins in *C. jejuni*. Page et al. (21) have found porins in *C. jejuni*, and they noted that the best β-lactam agents against this species were imipenem, ampicillin, amoxicillin, and cefpirome and attributed this to the dipolar ionic charges of these compounds, as noted by Nikaido (18). The lack of permeability of *C. jejuni* outer membranes to penicillin G could be related to the monoanionic charge of this agent (21). The exceptional bulky side chain of piperacillin may explain its poor penetration into *C. jejuni*, as in *E. coli* K-12 (29). Our results also suggest no role of this β-lactamase in resistance to cephalosporins. Further studies are needed to delineate the importance of the permeability barrier and penicillin-binding proteins in the resistance of *C. jejuni* to β-lactam agents.

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